EFFECTS OF TIME AND TEMPERATURE
as determined by viscometric methods

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THE FDA RECOMMENDS and the California Agricultural Code requires that the milk supply be continuously screened for somatic cell number. Milk with counts over 1.5 million cells per milliliter is not acceptable. The number of somatic cells in milk from individual cows is an indication of udder health. A somatic cell count in the range of 30,000 to 300,000 per milliliter is currently considered normal; a count of 500,000 or more per milliliter is indicative of mastitis or other udder abnormalities.

Several screening tests for somatic cell content have been developed which are inexpensive, quick, and require very little operator training. They usually employ some characteristic of the somatic cell. One such method measures the degree of viscosity produced when DNA (deoxyribonucleic acid) is released by chemical reagents such as alkylaryl sulphonate. Another tests the ability of the catalase from the cell to decompose hydrogen peroxide. Confirmatory tests entail expensive equipment, skilled labor, and more time, but they result in accurate cell counts. Confirmatory tests include Direct Microscope Somatic Cell Counts (DMSCC); Optic Somatic Cell Counts (OSCC); tests using electronic methods, such as the Coulter Counter; and chemical measurement of DNA.

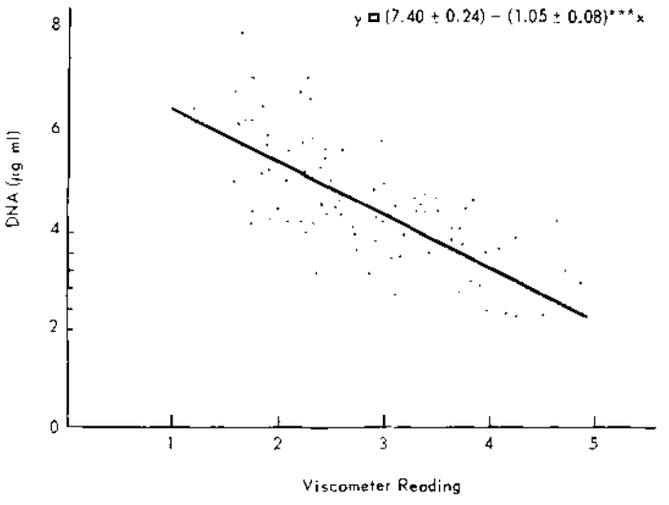
The California Mastitis Test (CMT) is used in California officially by the county health departments to screen farm bulk tank milk, and also by local Dairy Herd Improvement Association (DHIA) supervisors to screen cows monthly, as an indication of herd health. Use of DHIA data by University animal scientists for evaluating the effect of dairy equipment on udder health has led to equipment modifications in California's dairies. Although the CMT program and ensuing research have resulted in improved herd health and higher milk quality statewide, the CMT is not without limitations. For example: (1) CMT readings vary between and within individual readers and samples. (2) CMT scores represent a rather large and overlapping range of somatic cell content. (3) CMT scores change as the milk gets older.

Researchers continue developing methods and instrumentation which will assist in reducing the variability and improving the accuracy of screening tests. One such instrument, developed and tested in New Zealand and described in several papers by W. G. Whittlestone et al., is the Ruakura Rolling Ball Viscometer (RRBV). This instrument, like the CMT, tests viscosity. One part of milk is combined with two parts of a 2 percent solution of a secondary alkyl sulphate. This mixture of milk and reagent is gently poured into the viscometer tube, which is marked on a scale of 0 to 10. A synchronous motor driving a controlling cam is activated; after a 20-second holding period, the tube tips to a 25° angle for a predetermined (adjustable) period of time, allowing the steel ball to descend to the bottom of the tube. When the tube has automatically returned to the horizontal starting position (after about 3.5 seconds) the ball moves partly up the tube, coming to rest at some position on the scale. The distance the ball moves is inversely proportional to the viscosity of the mixture; that is, the higher the reading, the lower the viscosity.

In 1972, Whittlestone and Milne compared somatic cell counts obtained with the Ruakura Rolling Ball Viscometer to those obtained by DNA determinations. The cell count regression equation was calculated as: (Cell Count) $y = (7.40 \pm 0.24) - (1.05 \pm 0.08)^{***} x$. (See the graph.) In the same year, Leonard began studying the internal reliability of the Ruakura Rolling Ball Viscometer method, examing variation in readings on the same sample by one operator, and variability between operators. No measurable variability was noted between readings of different operators; the coefficient of variation within a sample was 16.4 percent. Viscosity was affected by time and temperature. Viscosity became lower over a period of time at room temperature, and freezing the milk samples resulted in a complete loss of viscosity; potassium dichromate was of little value when used to preserve viscosity. Kroger and Jasper, using the Wisconsin Mastitis Test, earlier had shown substantial changes in viscosity (and, therefore, in correlation of scores to cells) with changes in time and temperature.

Our objectives in this study were: (1) to evaluate time and temperature effects on the viscosity of milk samples with a known somatic cell content, (2) to consider the possible effects of these variables on somaic cell counts obtained from tests measuring degree of viscosity, and (3) to compare the accuracy of the Ruakura Rolling Ball Viscometer with that of the California Mastitis Test.

SOMATIC CELL COUNTS : RUAKURA ROLLING BALL VISCOMETER READINGS COMPARED TO DNA DETERMINATIONS



Procedure

The Ruakura Rolling Ball Viscometer (RRBV) was employed to measure the effect of time and temperature on the viscosity of milk samples collected and prepared each morning of the test.

Fresh milk samples with a CMT 2 reaction were collected from a Tulare County dairy farm and immediately taken to the D.C.C.A. laboratory of Tulare, California and tested in the RRBV.

ON THE SOMATIC CELL CONTENT OF MILK

Each sample was divided into four subsamples in the laboratory, and fresh pasteurized milk was added to obtain four samples of different cell content (table 1). Each of the four samples was again subdivided and the cell count for each subsample was determined by Direct Microscope Somatic Cell Count (DMSCC) as outlined by the National Mastitis Council, and electronically with the Coulter Counter (table 2). Each of these samples was thoroughly mixed and subdivided into 50 milliliter lots to minimize the effect of hourly agitation of the total sample for the duration of the test. Half of these samples were refrigerated (approxmately 5°C) and half retained at room temperature (approximately 23°C). A subsample was randomly selected from each lot every two hours; tested by the standard CMT procedure; three viscometer readings were taken; milk films were made for Direct Microscope Somatic Cell Counts; and Coulter Counter measurements were made at the beginning and at the conclusion of the trial.

TABLE 1. VISCOMETER, COULTER COUNTER, DMSCC AND CMT SCORES ON INITIAL MILK SAMPLES

	Average Reading for Each Sample			
Method	1	2	3	4
*Viscometer	2.375	3.929	2.429	3.625
Coulter Counter	2,210,000	1,189,000	2,124,000	1,320,000
DMSCC	2,004,000	1,247,000	1,960,000	1,347,000
CMT Score	2	2	2	2

TABLE 2. SUBSAMPLE CELL CONTENT, COUNTED BY DIFFERENT METHODS AT COMMENCEMENT OF TRIAL, CMT SCORES AT BEGINNING AND END OF TRIAL

	INITIAL READINGS							
	COULTER COUNTER		DMSCC		CMT 0 Hours		CMT 12 Hours	
Sample No.	23 C	5 C	23 C	5 C	23 C	5 C	23 C	5 C
	million cells		million cells					
1	2.211	2.209	2.030	1.978	2	2	2	2
2	1.170	1.208	1.295	1.400	2	2	1	1+
3	2.141	2.107	2.083	1.837	2	2	2-	2
4	1.303	1.339	1.470	1.225	2	2	1	2-

Warm barns

A second experiment was initiated to study the effect of holding milk at temperatures of a warm summer milking barn, the laboratory, and the refrigerator, to estimate the effect of sample temperature on viscosity.

The procedure followed was essentially the same as for the preceding test, except that one sample of fresh raw milk with a cell content of 2,244,800 cells per ml was obtained and divided into five equal portions—each of which was again subdivided into ten 50 ml subsamples. The cell content of each 50 ml subsample was determined by DMSCC. The five lots of ten subsamples were numbered from one to five and randomly chosen for each treatment. Lots 1 and 2 were held in a water bath at approximately 41°C; viscosity of Lot 1 was determined at 41°C, while a subsample from Lot 2 was lowered to room temperature before the viscosity was taken. Lot 3 was held at room temperature, and its viscosity read at room temperature. Lots 4 and 5 were refrigerated at about 5°C until tested. The subsample from Lot 4 was brought to room temperature before taking the viscosity, while a subsample from Lot 5 was read at the lower temperaure.

Results

Cell counts of each primary milk sample, as measured by the Coulter Counter and Direct Microscope Somatic Cell Count, and by two indirect methods (CMT and RRBV) were used as the basis of this experiment (table 1).

The results from the Coulter Counter and DMSCC appeared to be in excellent agreement, and the viscometer reading seems to be correlated to the cell content given. The CMT scores appear to be satisfactory. However, samples 2 and 4 should, by definition, have a CMT 1 score (CMT 2 = 1,500,000 – 3,500,000 cells per ml) and perhaps were scored too high.

The data in table 2 summarizes the cell counts of the original samples following their division into subgroups. As in table 1, the counts were in excellent agreement. The CMT scores for samples 2 and 4 are questionable, as they do not fit the definition for a CMT 2 score and may reflect a reader bias; however, the results do indicate a loss in reactivity with time. A highly significant relationship existed between viscosity of the sample with storage temperature ($F = 22.64^{***}$) and time ($F = 5.12^{***}$) (table 3).

Samples held at room temperature began showing reduced viscosity within 6 hours; those held at 5°C maintained their initial viscosity throughout the 12-hour test period. At the end of 24 hours, the samples held at 23°C were beyond the scale of the viscometer (10), while the samples held at 5°C appeared to have little, if any, change in viscosity from 12 hours earlier. The samples were measured at the end of a 24-hour storage period. It was noted that samples at 23°C had no measurable viscosity on the viscometer scale used. The samples held at 5°C had a reading of 3.5. The significance of this reading is not known at this time. Leonard noted similar changes in refrigerated samples stored for 24 hours. The results of the second trial are summarized in table 4. As in the first experiment, the effect of storage temperature was highly

TABLE 3. EFFECT OF TIME AND TEMPERATURE ON VISCOSITY AS MEASURED BY ROLLING BALL VISCOMETER

	Temperature of Stored Samples		Average
Hours	23 C	5 C	Both 23 C & 5 C
0	2.125	2.250	2.188
2	3.313	2.375	2.844
4	2.625	2.063	2.344
6	3.250	2.563	2.906
8	3.688	2.438	3.663
10	4.875	2.875	3.875
12	6.188	2.625	4.406
Average	3.723	2.455	

NOTE: The above data is based on averages of the samples. A within sample error C.V. of 21.8 percent was calculated.

TABLE 4. ROLLING BALL VISCOMETER READINGS AT THREE DIFFERENT STORAGE TEMPERATURES

	SAMPLE				
Initial	1	2	3	4	5
D.M.S.C.C. (Million)	2.123	2.224	2.205	2.045	2.293
Hour Held at Storage	41°/41°*	41°/23°	23°/23°	5°/23°	5°/5°
	(Centigrade)				
0	2.5	2.5	2.5	2.5	2.5
1	3.0	2.5	2.3	2.8	2.0
2†	2.5	2.0	1.5	1.3	1.3
3	4.3	2.0	1.5	1.5	1.5
4	4.3	4.0	2.3	2.3	2.0
5	6.0	5.8	3.5	1.8	1.8
6	5.5	6.0	2.8	2.3	1.5
7	5.8	4.75	3.5	2.3	2.0
8	7.3	7.0	3.8	1.8	1.8
Average	4.6	4.1	2.6	2.1	1.8

NOTE: The above data are based on averages of the readings of each subsample. A within sample error C.V. of 16.4 percent was calculated.

* Storage temperature/sample temperature at time of reading.

† Changed to a newly made reagent. All statistics an average of two measurements.

significant (F= 14.18***) as was storage time (F =3.87***).

The viscometer readings were made to the nearest half digit. The data indicate that samples held at the higher temperatures (41°C) began losing viscosity 2 to 3 hours after the experiment was initiated, those at room temperature began deteriorating at the 5th hour, while the refrigerated samples seemed to hold up throughout the experiment. Although the data were not calculated to determine the difference between lots, it did appear that the testing temperature had a slight effect upon the viscosity reading.

Storage temperature was extremely critical in its effect upon viscosity. The higher the storage temperature, the more rapidly sample viscosity is destroyed. At the other extreme, freezing was equally destructive as was the addition of potassium dichromate. Refrigeration of the milk sample gave consistently good results within a 24-hour time period, as measured by Rolling Ball Viscometer and CMT.

The precision of all gel viscosity tests, such as CMT and Wisconsin Mastitis Tests, are affected by time × temperature. Estimation of cell content of milk by gel viscosity methods without complete knowledge of how each milk sample was collected and stored, and without compensatory adjustment in interpretation, are subject to considerable error and are unsatisfactory for milk quality control.

The Ruakura Rolling Ball Viscometer lends itself to less subjective readings than CMT methods presently in use in California, resulting in greater agreement between readers testing the same sample. A relatively low C.V. 16 to 21 percent within sample variability was noted and could be lowered if reading procedure was altered to the nearest full digit reading, rather than the nearest estimated half digit. Further work is required to determine the full value of the Rolling Ball Viscometer in the future administration of cell count determinations.

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ALFALFA DAMAGE BY JACKRABBITS IN THE SOUTHERN CALIFORNIA DESERTS

Jackrabbits are significant threats to alfalfa production only when their population density is high, usually in drought periods preceded by years of plentiful rainfall. Jackrabbits living near alfalfa fields do not usually depend solely on alfalfa for nutrition, but individuals may consume up to 65 lbs dry alfalfa per year when desert forage is unsuitable. Observations indicate that hares may travel over two miles at night to reach fields. Fencing fields with poultry wire offers complete control.

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ALFALFA PRODUCTION contributes significantly to arid-land agriculture in southern California. During 1973, San Bernardino County alone produced alfalfa valued at over $5,000,000. Jackrabbits (*Lepus*) are conspicuous crop depredators in this area, periodically reaching epidemic proportions, and causing loss of entire cotton and alfalfa crops and destruction of range forage. Despite this economic loss, little work has been done to quantify crop damage due to jackrabbits.

To measure jackrabbit impact on alfalfa, detailed knowledge of the structure and behavior of the jackrabbit population must be obtained. It is important to know that jackrabbits infest alfalfa fields only at night, traveling some distance from the surrounding desert to consume alfalfa. Population density and the distance traveled by jackrabbits for food are important, as is the seasonal variation in impact arising from the changing nutritional needs of the jackrabbit population. As desert grasses and wildflowers dehydrate in late spring, the jackrabbit population is forced to rely more on the alfalfa fields for water and nutrition. During drought years jackrabbits may accumulate around fields.

To measure seasonal differences in nutrient supplementation and hare population density, jackrabbit use of agricultural areas near Barstow, California, was observed from winter through summer 1974. Succulent sources of vegetation apart from irrigated areas are not available from June through January at the study sites, where precipitation averages 2½ inches per year.

The Lenwood-Hinkley agricultural area and the Sun and Sky Golf Course, both about seven miles west of Barstow, were visited periodically at about 10 p.m., to monitor the total number of hares consuming grass or alfalfa. Jackrabbit use of the numerous ranches in the Lenwood-Hinkley area never exceeded three animals per night, but visibility was a problem when the alfalfa was tall. The golf course observation site afforded unimpeded visibility, and here jackrabbit presence reflected the abundance of succulent desert vegetation. Spring annuals were in full bloom from mid-April to early May, during which time no hares were seen at either the alfalfa fields or the golf course (graph 1).

Beginning in May 1974, daytime counts were made of hares living next to alfalfa fields at the Lockhart Ranch. These 3000-acre alfalfa fields are 23 miles northwest of Barstow near Harper dry lake. Early in the morning, two observers walked through the study area and noted the total number of hares seen. Flushing distance averaged 50 yards. The number of jackrabbits spotted was converted to density on a per acre basis. During May through August, a total distance of 33 miles was traversed. Data collected at Lockhart reveal population accumulations were dependent upon distance to fields and the vegetation type in the area. Within one mile west of Lockhart, in a